———. 1935. (Poales) Poaceae (pars). N. A. Fl. 17(6): 419–482. (See pp. 431–432.)
Johnston, I. M. 1943. Plants of Coahuila, Eastern Chihuahua, and adjoining Zacatecas and Durango, II. Jour. Arnold Arb. 24: 375–421.
Jones, M. E. 1912. New species and notes. Grass notes. Contr. West. Bot. 14: 1–21.
Lindgren, Waldemar. 1890. Notes on the geology and petrography of Baja California, Mexico. Proc. Calif. Acad. Sci. II. 3: 25–33.
Moran, Reid. 1952. The Mexican itineraries of T. S. Brandegee. Madroño 11(7): 253–262.
Nelson, Edward W. 1921. Lower California and its natural resources. Mem. Nat. Acad. Sci. 16(first Memoir): 1–171. pl. 1–35.
Niles, Cornelia D. 1925. A bibliographic study of Beauvois' Agrostographie. Contr. U. S. Nat. Herb. 24(6): 135–214. + index.
Setchell, William A. 1926. Townshend Stith Brandegee and Mary Katherine (Layne) (Curran) Brandegee. Univ. Calif. Publ. Bot. 13(9): 155–178. pl. 13, 14.
Scribner, F. Lamson & Effie A. Southworth. 1890. The true grasses. Translated from Hackel, Eduard. Die natürlichen Pflanzenfamilien. New York: Henry Holt & Co., viii + 228. illus.

## PHYTOSEROLOGY VERSUS GENEALOGY IN ZEA MAYS[1]

J. F. Davidson and T. L. Thompson

For many years the ideas of phytoserology, as expounded by Mez (1936) and by Chester (1937), have intrigued the senior author as being potentially highly significant in the field of plant taxonomy. Discussions with the junior author, and with his colleague, Dr. Warren Engelhard, stimulated a desire to attempt some phytoserological tests. While it is obvious from the literature that phytoserology has yielded taxonomic data, (i.e., data which give evidence of proximity of relationship), there is no assurance that these data are valid. In short, do the data obtained from serological studies accurately reflect the genealogies of the individuals in question? This was our problem.

In order to set up a test for the validity of phytoserology in taxonomy, it was necessary to find plants of *known* genetic (genealogical) relationships. It was felt that checking members of the same genus against members of other genera in the same plant family, or against members from other families, would not be accurate enough, since such relationships are *assumed,* not definitely known. It is true that such assumptions are based upon many data, and are probably valid, but the authors wished to test definite, not probable, genealogies. With the thought that *Zea Mays* had been bred for many years, the authors approached the corn breeders to see if they could obtain some strains of known genealogy. Many of their stocks were of *presumed* closer or more distant relationship, but the genealogies of some were *known.*

Through the gratefully appreciated cooperation of the Department of

[1] This work was financed by a grant from the University of Nebraska Research Council, whose cooperation is hereby gratefully acknowledged.

Agronomy of the University of Nebraska, we obtained six strains of corn in which the genealogies were known. In the interest of simplicity, the agronomists selected sample No. 1 as the reference sample, so that the others were more or less distantly related to No. 1. We arranged with the agronomists that after the serological relationships of the six samples had been determined, a check would be made against the recorded genealogies of the samples involved.

While the principles of serology are available in any standard textbook, such as Boyd (1943) or Rabat and Mayer (1948), the senior author has considered it advisable, in the interest of completeness, to include the following resumé of the serological principles as they apply to plant taxonomy. Furthermore, since the great majority of taxonomists are not familiar with the methodology, it is presented here in considerable detail.

The basis for phytoserology rests in the thesis that although the total protein constitution of a plant is antigenically different from that of any other plant, certain proteins are found in common in closely related individuals. Although each plant specimen is considered to possess some proteins that are peculiarly its own as an individual, still, genetic processes pass common proteins along to their offspring. Thus, those plants which are more closely related genetically are presumed to have a greater number of proteins in common.

A protein, when injected into the blood stream of a laboratory animal, may stimulate the formation of *antibodies* in the serum. The injected protein is termed the *antigen*. Injections of the same protein at frequent intervals may increase the concentration of the antibodies in the blood serum. If, after a "rest from injection" period, the serum is withdrawn from the animal and the original protein (antigen) is added to the serum, the anti-bodies present in the serum react with the antigen. In some instances, this reaction may express itself visually as agglutination or precipitation. In other instances it is necessary to employ "indicator" systems to detect the reaction. If the proteins are efficient as antigens, and if they are present in sufficient amounts, either the serum or the antigen may be greatly diluted (1:10,000) and still give a discernible reaction. Thus, unknown antigens may be checked against the antibodies present in the serum by making a series of dilutions for each unknown antigen, adding a standard amount of serum, and seeing at what dilutions the reaction is apparent. The greater the dilution showing a reaction, presumably the greater the number or amount of common proteins.

For antigen injection, it is desirable to employ an isotonic dilutent to avoid possible shock to the experimental animals. Thus, antigens which are insoluble in the dilutent used are not available for testing. The most common dilutent employed is physiological saline solution. However, when plant materials are extracted in a dilutent they frequently contain substances which are toxic to the test animals. Such toxins are commonly removed by one or several pre-extractions with some other solvent, such as ethyl alcohol or sulphuric ether. The solutions resulting from such pre-

extractions are discarded, and the plant material is then extracted in the physiological dilutent.

In certain tests in immunology it is desirable to inject either a single protein or a small number of proteins. Hence, the original extract is normally purified by successive pre-extractions with various organic solvents, thus eliminating the undesirable fractions.

However, in taxonomic work, there is no method of predetermining which proteins, if any, characterize the various phyletic lines. It is preferable, therefore, to have both the maximum amount and maximum number of proteins present. For this reason, in taxonomic work it is desirable that pre-extraction procedures, as shown in Chart 1, should be kept to a minimum.

Chart 1. Sequence of Pre-extraction Procedures

| Proteins in original plant | A* | Proteins lost in alcohol | Proteins lost in ether | Proteins remaining after pre-extraction in alcohol and ether |
|---|---|---|---|---|
| | Proteins Soluble in Saline | | | |

*A = Proteins insoluble in saline.

From this chart it can be seen that not all the proteins in the plant are necessarily soluble in saline. We have no method of determining or testing fraction A. The maximum we can test is the amounts or kinds present in the saline solution. Each succeeding pre-extraction reduces the number and/or kinds of protein tested. If the characteristic proteins of a genealogical lineage are not present in the antigen, the serological results cannot be indicative of the lineage.

## Procedure

Preparation of Antigens. Corn was selected for the experimental plants because of the *known* genealogies. Since the corn fruit is a genetic mixture, as far as tissues are concerned, with fruit coat which is strictly maternal, endosperm which is largely maternal, and embryo which is half maternal and half paternal, only the young plant, derived from the embryo was used. It was felt that the use of the grain itself would introduce probable errors.

The six samples of corn were planted in vermiculite, 50 plants per flat, one flat per sample. They were harvested after 38 days and each plant was uprooted and the whole plant was cut up with shears into one-fourth to one-half inch pieces. Any remnants of the original grain were discarded. From each sample, 400 grams of plant material were used. This material was pulverized, 25 grams at a time, in 200 ml. of physiological saline in a Waring Blendor for 45 seconds. The resultant mixture was squeezed through cotton toweling and the debris was discarded. (The authors now feel that it might have been wise to have allowed the mixture to extract overnight in a refrigerator before the above rough filtration, as a possible method of increasing the protein concentration.)

The extractions were held overnight at 5°C. and were then centrifuged 30 minutes at 2,000 r.p.m. After centrifuging, the residue was discarded and the supernatant was passed through a sterile Seitz filter. The filtered extract was transferred to 200 ml. rubber-capped serum bottles, and 1:10,000 Merthiolate was added as a preservative. Extracts were stored at 0°C. until needed.

Nitrogen determinations were made by the colorimetric method of Miller and Miller (1948). Prior to the serological experiments, each sample was adjusted to contain 0.16 mg. of nitrogen per ml. The six samples varied as shown:

Table 1. Variation in Nitrogen Content of Extract

| Sample No. | N in mg./ml. | Diluted to |
|---|---|---|
| 1 | 0.20 | 8 in 10 |
| 2 | 0.40 | 8 in 20 |
| 3 | 0.50 | 8 in 25 |
| 4 | 0.16 | undiluted |
| 5 | 0.24 | 8 in 12 |
| 6 | 0.18 | 8 in 9 |

Test Animals. Preliminary experiments indicated that mature rabbits did not produce a good antibody titer in response to the corn proteins. Good titers were, however, obtained when rabbits two months of age were employed.

Injection of Antigen. The animals were injected every fourth day with the amounts of plant extract No. 1 shown in Table 2. Four injection routes were employed to determine the most efficient in building up the antibody titer (see Table 4). These were: intravenous, intraperitoneal, intramuscular, and subcutaneous.

The intravenous and intraperitoneal routes proved to give the highest titers, and since these titers were both approximately equivalent, the intraperitoneal route was employed as being the easier to administer.

Tube Agglutination Tests. For preliminary titrations, 5 ml. of blood were withdrawn from the terminal ear vein, and allowed to clot at room

Table 2. Injection Routes and Quantities

| Day | Millimeters injected | | | |
|---|---|---|---|---|
| | Intravenous | Intra-peritoneal* | Intra-muscular* | Subcutaneous |
| 1 | 0.2 ml. | 0.5 ml. | 0.5 ml. | 0.5 ml. |
| 4 | 0.4 | 1.0 | 1.0 | 1.0 |
| 8 | 0.8 | 2.0 | 2.0 | 2.0 |
| 12 | 1.0 | 2.0 | 2.0 | 2.0 |
| 16 | 2.0 | 5.0 | 5.0 | 5.0 |
| 23 | Animals bled, preliminary titer determined. | | | |
| 37 | 2.0 | 5.0 | | |
| 51 | Final bleeding. | | | |

*Addition of an adjuvant (mucin) did not enhance the antibody titer.

temperature. The clots were broken, and the serum was separated by centrifugation at 3,000 r.p.m. for 30 minutes. The serum was serially diluted with physiological saline as follows: 0, 1:2, 1:4, 1:8 up to 1:256. One ml. of each dilution was pipetted to a serological tube, one ml. of antigen diluted 1:10 was added, and the tubes were shaken vigorously. The tubes were then incubated at 37°C., and observed at intervals over an 8-hour period. A reciprocal test was also set up using antigen dilutions and a constant amount of a 1:2 dilution of serum.

Ring Precipitation Tests. Small amounts of undiluted serum were introduced into 5 cm. micro tubes with a bore of 2 mm. By means of a capillary pipette, each antigen dilution was carefully overlaid on the surface of the serum. The tubes were incubated at 37°C., and checked at intervals for the characteristic precipitin ring at the interface.

Titers obtained from these two methods were disappointingly low. In no instance did the titer exceed 1:32.

In order to determine the effect of incubation temperature on agglutination, tubes were incubated at 5°C., 25°C., and 37°C. Incubation temperature was found to have no effect on the final titer.

It had originally been planned to employ agglutination tests, and to run cross-agglutinations to determine more accurately the relationship of the corn samples. However, in view of the low titers obtained, cross-agglutination reactions would have been of little value. Therefore, it was decided to check for the presence of complement-fixing antibodies.

Complement Fixation Reaction. The theory of complement fixation is predicated upon the following facts:

1. Complement, a protein, is always present in normal serum.
2. Certain immunological reactions require the presence of complement.
3. These reactions are not visible.
4. Hemolysin, an antibody produced by injecting sheep red blood cells (RBC) into rabbits, will lyse sheep red blood cells *only* in the presence of complement.

Where complement-fixing antibodies are produced, their presence is detected in the following manner. The serum is heated for 30 minutes at 55°C. to inactivate its complement, and some antigen plus an optimal amount of pure complement is added. This mixture is incubated to allow the antigen and complement-fixing antibodies to tie up the complement. Then anti-sheep hemolysin, in known amount, and sheep red blood cells are added and incubated for 30 minutes at 37°C. If all of the complement has been tied up by the antigen-antibody reaction, none will be available to react with the hemolysin to lyse the red blood cells. If only a small amount of complement-fixing antibodies is present, some of the complement will be available, and lysis will occur. The tube containing the lowest dilution of serum which shows complete lysis is taken as an indication of the titer.

Titration of Hemolysin for Optimal Amount. Using 1 ml. of 1:30 dilution of purified complement, a series of dilutions of hemolysin were added to determine the dilution that gave complete lysis in 30 minutes at 37°C. This amount of hemolysin is one unit. Two units were used in the final test.

Titration of Complement for Optimal Amount. Using two units of hemolysin, dilutions of complement were made to determine the dilution that gave complete lysis in 30 minutes at 37°C. This amount of complement is one unit. Two units of complement were used in the final test.

A further complication arising in the present work was due to the fact that the corn extracts apparently contained some substance which inactivated or destroyed the complement. This was controlled by diluting the antigen 1:1 and by adding sufficient complement to offset this reaction.

Table 3. Complement Fixation Test on Corn Samples

| Tube | Dilution of serum | Antigen* Diluted 1:1 | 2 units complem. 1/30 dil. | | Saline | Hemolysin 2 units | Red blood cells 3% | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0. | .25 ml. | 1.75 ml. | | 0. ml. | 0.25 ml. | 0.25 ml. | |
| 2 | 1:112 | | | | | | | |
| 3 | 1:24 | | | | | | | |
| 4 | 1:48 | | | | | | | |
| 5 | 1:96 | | | | | | | |
| 6 | 1:192 | | | Refrigerate 20 hours at 5°C. | | | | Incubate for 30 minutes at 37°C., and read. |
| 7 | 1:384 | | | | | | | |
| 8 | 1:768 | | | | | | | |
| 9 | 1:1536 | | | | | | | |
| 10 | 1:3072 | | | | | | | |
| Hemol. control | | 0 | 1.75 | | .75 | | | |
| Comp. control | | 0 | 1.75 | | 1.00 | 0 | | |
| Sensit. cont. | | 0 | 0 | | 2.50 | 0.25 | 0.25 | |
| Cell control | | 0 | 0 | | 2.75 | 0 | 0.25 | |
| Serum control | | 0 | 1.75 | | .75 | 0.25 | 0.25 | |

*The first ten tubes were repeated for each antigen.

Table 4. Results of Complement Fixation Test

| Antigen | Control | 1/12 | 1/24 | 1/48 | 1/96 | 1/192 | 1/384 | 1/768 | 1/1536 | 1/3072 | Titer |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | +++ | — | — | — | — | — | +— | +— | +— | (+++) | 3072 |
| 2 | +++ | — | — | — | — | +— | (+++) | +— | +++ | +++ | 384 |
| 3 | +++ | — | — | — | — | +— | +— | (+++) | +++ | +++ | 768 |
| 4 | +++ | — | — | — | — | — | — | +— | +— | (+++) | 3072 |
| 5 | +++ | — | — | — | — | +— | +— | +— | (+++) | +++ | 1536 |
| 6 | +++ | — | — | — | — | +— | (+++) | +++ | +++ | +++ | 384 |
| Hemol. | +++ | | | | | | | | | | |
| Comp. | — | | | | | | | | | | |
| Sensit. | — | | | | | | | | | | |
| RBC | — | | | | | | | | | | |
| Serum | +++ | | | | | | | | | | |

+++ complete lysis
— complete inhibition
( ) titer

Best results were obtained when the antigen and serum were incubated with the complement at 5°C. for 20 hours.

After the preliminary titer from the injection of antigen had attained a value of over 1:3,000, the other antigens were tested as shown in Table 3. The results of this test appear in Table 4.

From the above it would appear that, as far as similarity of proteins is concerned, samples 1 and 4 are most closely related, and that the remainder are, in order of similarity, 5, 3, with 2 and 6 being equally distant from 1. This might be shown graphically as:

1 5 3 2
4 6

## Discussion

After the above results had been obtained, the genealogy of the samples was requested from the Department of Agronomy. The samples represented the following plants:

1. Dent corn N 6
2. Dent corn hybrid L 289 × I 205
3. Dent corn 38-11
4. Pop corn SA 24
5. Dent corn N 6 (from a sister plant to sample 1)
6. Dent corn hybrid L 289 × I 205 (from the same plant as 2)

The relationship of these plants, as given by the geneticists who know the genealogies is as follows: 1 and 5 are very closely related, being sister plants; next is the pure line, 3; then 2 and 6, being from the same plant; and most distantly related is the popcorn, sample 4. This can be shown graphically as:

1 5 3 2 4
6

It is interesting to notice that, with the exception of the popcorn (4), the serology reflects the genealogy quite well. While the serology was able

to distinguish between the sister plants (1 and 5), it was unable to distinguish between fruits from the same individual (2 and 6). These latter results would indicate that the methodology was sound, but because of the exception noted above, it would appear that serology of itself cannot be accepted as an adequate criterion of relationship. Within known groups, such as the dent corn samples utilized, it appears to be valid — at least from this limited series of experiments. As with most taxonomic tools, it would seem that serological data should be correlated with evidence from other fields to be significant.

Summary

Six samples of corn of *known* genealogy were tested by serological methods to determine whether or not the serological relationships were an accurate reflection of the genealogical relationships. The serological procedures are given. Results indicate that within the dent corns the serology did deflect the genealogical relationships, but the inability of serological techniques to distinguish between a dent corn and a popcorn demonstrates that serology of itself cannot be used as a valid clue to genealogical relationships in every case.

Department of Botany, and
Department of Bacteriology,
University of Nebraska, Lincoln

Literature Cited

Boyd, W. C. 1947. Fundamentals of Immunology, 2nd Ed. Interscience Publishers, London, England. 503 pp.

Chester, K. S. 1937. A critique of plant serology. Quart. Rev. Biol. 12: 19–46, 165–190, 294–321.

Kabat, E. A. and M. M. Mayer. 1948. Experimental Immunolchemistry, 1st Ed. Chas. C. Thomas, Chicago. 567 pp.

Mez, C. 1936. Morphologie and Serodiagnostik. Bot. Arch. 16: 1–23.

Miller, G. L. and E. E. 1948. Determination of nitrogen in biological materials. Analyt. Chem. 20: 481–488.

## JOSEPH BURKE IN 1853

R. Kent Beattie

In her recently published work on the history of botanical exploration west of the Mississippi (McKelvey, Susan Delano. Botanical Exploration of the Trans-Mississippi West 1790–1850. Arnold Arboretum, pages 792–817. 1955), Mrs. McKelvey gives a very excellent and adequate account of the botanical explorations of Joseph Burke in western Canada and the western United States in the years 1843 to 1847. For years, little has been known by botanists about Burke's work except that he collected some plants around "Fort Hall" in what is now known as southern Idaho. We had learned that there was material about Burke at Kew, but no one